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Copper(I) Nitrosyls from Reaction of Copper(II) Thiolates with *S*-Nitrosothiols: Mechanism of NO Release from RSNOs at Cu

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Supporting Information

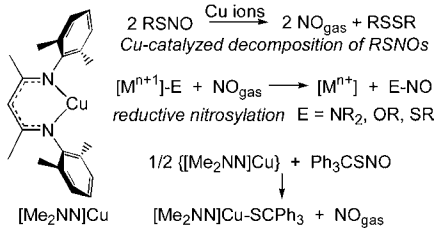
ABSTRACT: *S*-nitrosothiols (RSNOs) serve as ready sources of biological nitric oxide activity, especially in conjunction with copper centers. We report a novel pathway for the generation of NO within the coordination sphere of copper model complexes from reaction of copper(II) thiolates with *S*-nitrosothiols. Reaction of tris(pyrazolyl)borate copper(II) thiolates ^{iPr2}TpCu−SR (R = C_6F_5 or CPh_3) with ^{t}BuSNO leads to formation of ^{iPr2}TpCu(NO) and the unsymmetrical disulfide RS−S^{t}Bu. Quantum mechanical investigations with B3LYP-D3/6-311G(d) suggest formation of a κ^1-N−RSNO adduct ^{iPr2}TpCu(SR)(R′SNO) that precedes release of RSSR′ to deliver ^{iPr2}TpCu(NO). This process is reversible; reaction of ^{iPr2}TpCu(NO) (but not ^{iPr2}TpCu(NCMe)) with $C_6F_5S-SC_6F_5$ forms ^{iPr2}TpCu−SC_6F_5. Coupled with the facile, reversible reaction between ^{iPr2}TpCu(NO) and C_6F_5SNO to give ^{iPr2}TpCu−SC_6F_5 and 2 equiv NO, we outline a new, detailed catalytic cycle for NO generation from RSNOs at Cu.

S-nitrosothiols, RSNOs, serve both as ready reservoirs of NO activity and active agents in the post-translational modification of proteins through *S*-transnitrosation of cysteine SH residues.[1] Low-molecular weight RSNOs such as *S*-nitrosoglutathione (GSNO) are present at submicromolar concentrations in the plasma[2] and exhibit a number of beneficial physiological effects[1] such as protection against myocardium[3] and lung/airway[3] injuries. Due to the modest RS-NO bond strength (20−32 kcal/mol),[4] RSNOs serve as air-stable reservoirs of NO.

Offering the prospect of controlled, triggered NO release in biological environments, copper ions are effective in catalyzing RSNO decomposition to NO_{gas} and disulfides RSSR (Scheme 1).[5] CuZnSOD which represents the most abundant source of copper in red blood cells releases NO from GSNO.[6] NO release may be inhibited with neocuproine, a Cu^+-specific chelator.[6] Taking advantage of endogenous RSNOs in the plasma, Cu^{2+} ions embedded into medical polymers can serve as long-lived NO generating devices.[7] Thus, copper complexes starting in both the Cu(I) and Cu(II) oxidation states can be effective for release of NO from RSNOs.

We recently reported the formation of NO in the reaction of an electron-rich β-diketiminato copper(I) complex [Me_2NN]Cu with Ph_3CSNO to give the copper(II) thiolate

Scheme 1. Copper-Mediated Reactions with *S*-Nitrosothiols



[Me_2NN]Cu−SCPh_3 (Scheme 1).[8] Release of NO from E−NO at a Cu(I) center is the microscopic reverse of reductive nitrosylation, which occurs at Cu(II) centers [Cu^{II}]−E with concomitant formation of the organonitroso compound E−NO.[9] Related reductive nitrosylation reactions involving the formation of O−NO bonds has been observed in laccase[10] and other enzymes.[11] The formation of N−NO bonds with NO is a common pathway in reaction of copper(II) amine complexes[12] and has been used as the basis for NO detection.[13]

Copper(II) thiolates are ubiquitous in biology, especially in "blue copper" type 1 Cu sites possessing strong absorbances near λ = 600 nm ($\varepsilon \approx 3000-4000$ M^{-1} cm^{-1})[14] that mediate electron transfer in a number of enzymes. Ceruloplasmin, the most abundant source of copper in the plasma (~1.5 − 3.1 μM),[15] contains three type 1 Cu sites, two with Met donors and one without. Coupled with the relatively high concentration of low molecular weight *S*-nitrosothiols (0.2 − 0.3 μM) in the plasma,[2] the possibility of interaction between copper(II) thiolates related to type 1 Cu with RSNOs is of interest, especially since ceruloplasmin has been shown to generate RSNOs from NO and thiols such as glutathione.[16]

In this study, we employ copper(II) thiolates supported by tris(pyrazolyl)borates, which have been used as structural[17] and spectroscopic[17,18] models for type 1 Cu sites. These biological copper centers feature two histidine N-donors, an anionic cysteine S-donor in addition to a weak donor such as methionine that results in only a slightly distorted trigonal environment at copper. X-ray structures of TpCu−SR species generally reveal two shorter Cu−N (**1**: 1.930(9), 2.037(9);[17a] **2**: 1.97(5), 2.03(4))[17b] and one modestly longer Cu−N (**1**: 2.119(8); **2**: 2.05(4)) distances with a relatively short Cu−S distance (**1**: 2.176(4); **2**: 2.12(2)).

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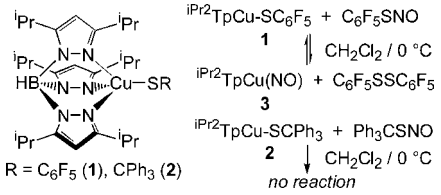
To examine the reaction between copper(II) thiolates and RSNOs, we employed the previously reported ^{iPr2}TpCu−SR (R = C_6F_5 (**1**) and CPh_3 (**2**)) which possess reasonable thermal stability. Reaction of **1** with C_6F_5SNO (prepared *in situ* from $[NO]BF_4$ and $TlSC_6F_5$) in CH_2Cl_2 (at 0 °C over 12 min) led to partial consumption of **1** (12%) as judged by the loss of the strong band of **1** at λ_{max} = 666 nm ($\varepsilon \approx 5900\ M^{-1}\ cm^{-1}$) with formation of a new band at λ_{max} = 495 nm. GC/MS and ^{19}F NMR analysis indicates formation of the disulfide $C_6F_5S-SC_6F_5$ in 18% yield (Scheme 2). Addition of the much larger *S*-nitrosothiol Ph_3CSNO to the bulky copper(II) thiolate ^{iPr2}TpCu−$SCPh_3$ (λ_{max} = 625 nm; $\varepsilon \approx 6600\ M^{-1}\ cm^{-1}$) gave no reaction under similar conditions.

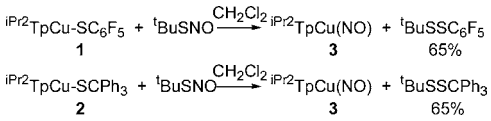
Scheme 2. Reactivity of ^{iPr2}TpCu−SR with RSNO



The new optical band at λ_{max} = 495 nm in the reaction of **1** with C_6F_5SNO is the copper(I) nitrosyl ^{iPr2}TpCu(NO) (**3**) which may be generated independently by reaction of ^{iPr2}TpCu with excess NO (λ_{max} = 495 nm ($\varepsilon \approx 960\ M^{-1}\ cm^{-1}$)); ν_{NO} = 1704 cm^{-1}) at −40 °C. While it is clear that ^{iPr2}TpCu(NO) (**3**) forms in the reaction of ^{iPr2}TpCu−SC_6F_5 and C_6F_5SNO, **3** is difficult to quantify because of its relative instability. Related tris(pyrazolyl)borate copper(I) nitrosyls TpCu(NO) feature labile binding of NO which may also disproportionate over time to TpCu(NO_2) and N_2O.[19] Nonetheless, addition of the disulfide $C_6F_5S-SC_6F_5$ to ^{iPr2}TpCu(NO) (**3**) (*in situ* generated at −40 °C in CH_2Cl_2) at 0 °C results in the formation of ^{iPr2}TpCu−SC_6F_5 (**1**) (68% yield; UV−vis) and C_6F_5SNO, indicating an equilibrium (Scheme 2). Monitoring over the temperature range −70 °C to −40 °C gives the thermodynamic parameters ΔH_r = −2.3(2) kcal/mol and ΔS_r = −14.5(8) for this reversible transformation (Figure S5).

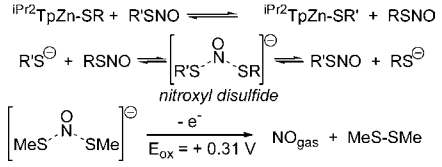
To better understand the fate of the *S*-residues in the reaction of ^{iPr2}TpCu−SR with *S*-nitrosothiols, we added the distinct *S*-nitrosothiol ^{t}BuSNO to **1** and **2** at 25 °C in CH_2Cl_2 (Scheme 3). In each case, the predominant S-containing product is the unsymmetrical disulfide RS−S^{t}Bu. Following the reaction of 1 equiv ^{t}BuSNO to ^{iPr2}TpCu−SC_6F_5 (**1**) by UV−vis spectroscopy results in the decay of **1** and formation of ^{iPr2}TpCu(NO) (**3**). ^{19}F NMR analysis following reaction in benzene-d_6 reveals that the unsymmetrical disulfide $C_6F_5S-S^tBu$ is the sole fluorine-containing product; the disulfide ^{t}BuS−S^{t}Bu accounts for the remainder of the ^{t}BuSNO employed. The reaction between ^{iPr2}TpCu−$SCPh_3$ (**2**) and ^{t}BuSNO proceeds similarly with loss of **2**, formation of **3**, and identification of Ph_3CS-S^tBu as the major new S-containing product with ^{t}BuS−S^{t}Bu as the remainder. A preliminary kinetic study via initial rates identifies that the reaction is clearly first order in ^{t}BuSNO when the initial ^{iPr2}TpCu−$SCPh_3$ concentration is held constant (Scheme S4).

Scheme 3. Reactivity of ^{iPr2}TpCu−SR with ^{t}BuSNO at 25 °C



The absence of the symmetric disulfides $C_6F_5S-SC_6F_5$ and $Ph_3CS-SCPh_3$ (<5%) in these reactions suggested that *S*-transnitrosation does not compete with Cu−NO and RS−SR bond formation. This is in contrast to the closely related zinc thiolates ^{iPr2}TpZn-SR′ which react with RSNO to cleanly undergo *S*-transnitrosation to give equilibrium mixtures of ^{iPr2}TpZn-SR and R′SNO (Scheme 4).[20] The later reaction appears to be a zinc-mediated transnitrosation of thiolate anions R′S^- with *S*-nitrosothiols RSNO which have been shown by experiment[21a] and theory[21b] to proceed via nitroxyl disulfide intermediates $[R'S(NO)SR]^-$ (Scheme 4).

Scheme 4. Reactivity of Thiolates with *S*-nitrosothiols



We used dispersion-corrected density functional theory (DFT-D3)[22] and *Gaussian 09*[23] to investigate the reactivity of Cu(II) thiolates with *S*-nitrosothiols. For computational efficiency, we considered ^{iPr}TpCu−SC_6F_5, a steric model of **1** that involves only the isopropyl substituent on each pyrazolyl ring that flanks the coordination pocket. Optimization results in two short and one long Cu−N distance (1.99 Å and 2.24 Å, respectively) and a Cu−S distance of 2.22 Å at the B3LYP/6-311G(d) level, in good agreement with the X-ray structure of **1**. Dispersion corrected energies suggest a slightly endothermic reaction between ^{iPr}TpCu−SC_6F_5 and C_6F_5SNO (ΔH_r = 1.2 kcal/mol) in accordance with the experimentally observed reversibility of the reaction (Scheme 2). The reaction of ^{iPr}TpCu−SC_6F_5 with ^{t}BuSNO is predicted to be slightly exothermic (ΔH_r = −2.1 kcal/mol). These are close to values measured by experiment (−2.3(2) kcal/mol and −1.7(1) kcal/mol, respectively).

We first considered the possibility of a nitroxyl disulfide anion $[RS(NO)SR']^-$ bound to a copper(II) center in ^{iPr2}TpCu(κ^2-SR(NO)SR′) along the reaction pathway. This was especially attractive, since theoretical studies on nitroxyl disulfides have suggested that these are rather unstable toward oxidation to NO and the corresponding disulfide (Scheme 4). For instance, the aqueous oxidation potential of [MeS(NO)-SMe]$^-$ anion was estimated as +0.31 vs NHE[21b] and the oxidation potential of ^{iPr2}TpCu(NCMe) is +0.64 V in MeCN.[24]

Quantum mechanical investigations on a small model system employing MeSNO with TpCu−SMe possessing the unsubstituted tris(pyrazolyl)borate ligand were performed at the B3LYP-D3/6-311+G(d) level of theory (Scheme 5). Formation of the symmetric nitroxyl disulfide intermediate TpCu(κ^2-MeS(NO)SMe) (**4**) is predicted to be exothermic with respect to the starting materials (ΔH_r = −14.2 kcal/mol and ΔG_r = 0.7 kcal/mol). The optimized structure of **4** shown in Scheme 5, however, suggests a sterically unfavorable orientation of the alkyl groups of the nitroxyl disulfide toward the Tp ligand, which is partly compensated by favorable dispersion interactions. Indeed, inclusion of the steric bulk around the reaction center using the steric model ^{iPr}TpCu revealed that

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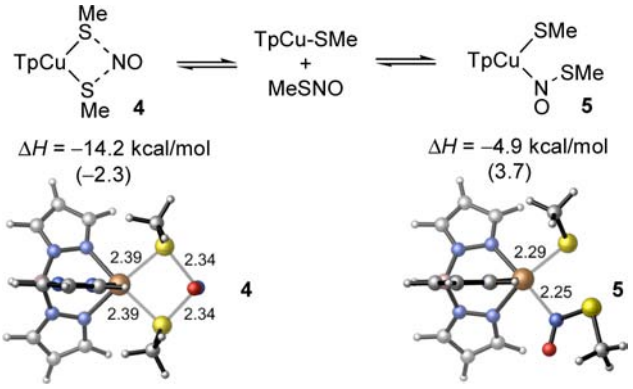
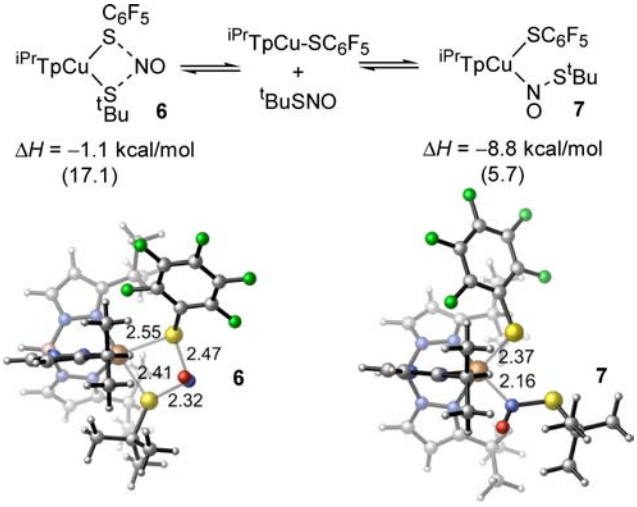
Scheme 5. Energetics of Reactions of Model TpCu−SMe with MeSNO Calculated with B3LYP-D3/6-311+G(d)[a]



[a]Non-dispersion corrected B3LYP enthalpies are given in parentheses.

formation of the corresponding nitroxyl disulfide **6** from ^{iPr}TpCu−SC_6F_5 and ^{t}BuSNO is sterically more challenging (ΔH_r = −1.1 kcal/mol and ΔG_r (298 K) = 12.5 kcal/mol; Scheme 6).

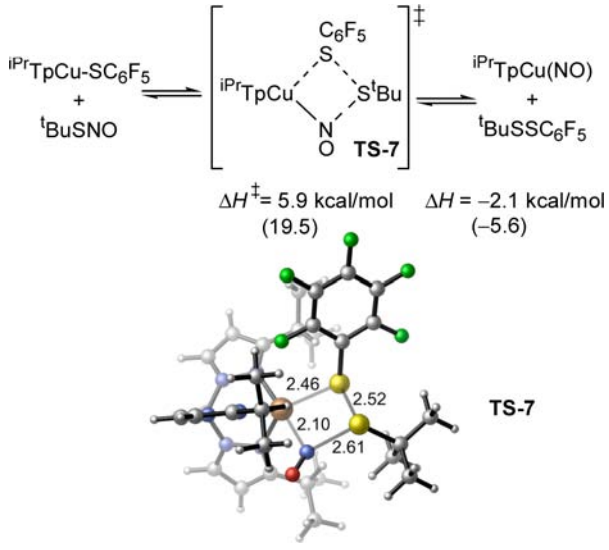
Scheme 6. Energetics of Reactions of Steric Model ^{iPr}TpCu−SC_6F_5 with ^{t}BuSNO Calculated with B3LYP-D3/6-311G(d)[a]



[a]Non-dispersion corrected B3LYP enthalpies are given in parentheses.

We also considered direct attack of an RSNO at the metal center that leads to a Cu−N interaction in TpCu(SMe)(κ^1-N(O)SMe) (Scheme 5). While this κ^1-N binding mode for MeSNO at a naked Cu^+ ion previously has been theoretically considered,[25] isolable complexes bearing *S*-nitrosothiols coordinated to transition metal ions are exceedingly rare and are limited to a few kinetically inert, low-spin Ir(III) species.[26] Although κ^1-N coordination of MeSNO to TpCu−SMe to give **5** (ΔH_r = −4.9 kcal/mol, ΔG_r = 7.4 kcal/mol) is predicted to be less favorable than **4**, we find that κ^1-N coordination of the much bulkier ^{t}BuSNO to ^{iPr}TpCu−SC_6F_5 in **7** is substantially more stable (ΔH_r = −8.8 kcal/mol, ΔG_r(298 K) = 5.3 kcal/mol) than its nitroxyl disulfide counterpart **6**. Unfavorable steric interactions at the Cu center in **6** between bulky thiolate *tert*-butyl and TpCu isopropyl substituents are significantly relieved by the κ^1-N binding mode of ^{t}BuSNO in **7**. The conversion of **7** to the corresponding TpCu(NO) and RSSR′ species proceeds smoothly via the transition state **TS7** with an activation enthalpy of 5.9 kcal/mol ($\Delta G^{‡}$ (298 K) = 19.4 kcal/mol) with respect to the starting materials (Scheme 7).

Scheme 7. Conversion of ^{iPr}TpCu(SR)(N(O)SR′) to ^{iPr}TpCu(NO) and RSSR′ Calculated with B3LYP-D3/6-311G(d)[a]



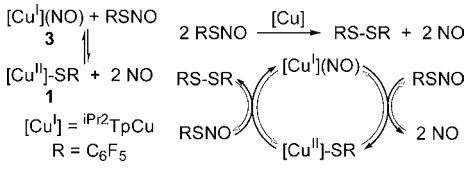
[a]Non-dispersion corrected B3LYP enthalpies are given in parentheses.

The concerted cleavage of the RS−NO and Cu−SR bonds with the simultaneous formation of the RS−SR bond in the κ^1-N−RSNO adduct ^{iPr}TpCu(SR)(R′SNO) as depicted in **TS7** accounts for the experimental formation of only unsymmetrical disulfides.

The dispersion energy correction terms calculated with DFT-D3 contributes 8−16 kcal/mol to the formation enthalpies of **4**-**7**. This clearly suggests the importance of dispersion interactions in stabilizing these intermediates and demonstrates the necessity of the accurate treatment of dispersion effects in these calculations.

The reaction of [Cu^{II}]−SR with RSNO to form [Cu^{I}](NO) and RS−SR closes a catalytic cycle for release of NO from *S*-nitrosothiols provided that copper(II) thiolates [Cu^{II}]−SR are formed upon reaction of copper(I) complexes [Cu^{I}] and RSNOs (Schemes 1 and 8). Reaction of ^{iPr2}TpCu(NO) (**3**)

Scheme 8. Copper-Catalyzed Release of NO from RSNOs



with 1 equiv C_6F_5SNO at −40 °C in CH_2Cl_2 leads to incomplete consumption of **3** with formation of ^{iPr2}TpCu−SC_6F_5 (**1**). Using the steric model ^{iPr}TpCu, calculations predict this reaction to be mildly favorable with ΔH_r = +1.9 kcal/mol and ΔG_r (298 K) = −6.7 kcal/mol. This reaction is reversible; addition of excess NO to ^{iPr2}TpCu−SC_6F_5 at −40 °C forms ^{iPr2}TpCu(NO) (**3**) (Scheme 8). Thus, addition of excess C_6F_5SNO to **1** or **3** at −40 °C results in the facile Cu-catalyzed

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decomposition to $C_6F_5S-SC_6F_5$ and NO_{gas} over 5 min, provided that the solution is gently bubbled with N_2 to remove NO_{gas} as it is formed. Although thermally sensitive around room temperature, this *S*-nitrosothiol shows little (<5%) decay in the absence of **1** or **3** at −40 °C in CH_2Cl_2.

Given the similarity between TpCu−SR models and type 1 Cu sites, these studies suggest that RSNOs could directly react with the Cu−SCys moiety at these biological copper centers. The constrained protein environment in which the type 1 Cu sites are embedded, however, could help resist the loss of a disulfide with formation of a $[Cu^I](NO)$ species. Since the SCys moiety is constrained by the protein structure, small molecule RSNOs would be much more freely diffusable than would a product disulfide. Nonetheless, type Cu sites in a number of enzymes such as ceruloplasmin[27] and ascobate oxidase[28] have been shown to reversibly react with NO itself. Future reports will describe our efforts at modeling the nature of the $[Cu^I](RSNO)$ intermediates formed upon addition of NO to biologically relevant $[Cu^{II}]$-SR complexes.

■ ASSOCIATED CONTENT

Supporting Information

Experimental, characterization, and calculational details. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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